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Major Histocompatibility Complex Class II (HLA-DRB and -DQB) Allele Frequencies in Botswana: Association with Human Immunodeficiency Virus Type 1 Infection

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Southern Africa is facing an unprecedented public health crisis due to the high prevalence of human immunodeficiency virus type 1 (HIV-1). Vaccine development and testing efforts, mainly based on elicitation of HIV-specific T cells, are under way. To understand the role of human leukocyte antigen (HLA) class II alleles in HIV pathogenesis and to facilitate HLA-based HIV-1 vaccine design, we analyzed the frequencies of HLA class II alleles within the southern African country of Botswana. Common HLA class II alleles were identified within the Batswana population through the molecular genotyping of DRB and DQB1 loci. The DRB1 allele groups DRB1*01, DRB1*02/15, DRB1*03, DRB1*11, and DRB1*13 were encountered at frequencies above 20%. Within the DQB1 locus, DQB1*06 (47.7%) was the most common allele group, followed by DQB1*03 (39.2%) and DQB1*04 (25.8%). We found that DRB1*01 was more common in HIV-negative than in HIV-positive individuals and that those who expressed DRB1*08 had lower median viral loads. We demonstrate that the frequencies of certain HLA class II alleles in this Batswana population differ substantially from those in North American populations, including African-Americans. Common allele groups within Botswana cover large percentages of other African populations and could be targeted in regional vaccine designs.

In most countries in southern Africa, human immunodeficiency virus type 1 (HIV-1) prevalence rates exceed 20% in the adult population (49). HIV-1 subtype C is the predominant HIV-1 genotype in southern Africa (37, 40, 52). HIV-1 subtype C is also the predominant and most rapidly spreading HIV-1 subtype in the world (11, 12, 39). The HIV-1 subtype C epidemic in southern Africa is characterized by a relatively high level of intrasubtype diversity (8, 35, 50). Virological and host factors may be at least partially responsible for the rapid expansion and predominance of HIV-1 subtype C in southern Africa and elsewhere. Host factors also clearly affect susceptibility to HIV infection and disease progression (44). Among the host factors that have been shown to affect susceptibility to HIV infection and disease progression are chemokine receptor polymorphisms, HIV-specific immune responses, and polymorphisms in molecules that play a key role in antiviral immune responses (9, 18, 22, 28, 43, 47). The frequencies of some of these host factors that influence HIV transmission and pathogenesis differ among populations according to ethnic or racial background (26), and alleles associated with susceptibility or resistance to HIV-1 may differ among racially distinct groups (13, 14, 42). It is largely unclear to what extent host genetic differences may account for the differential spread of HIV in the world, particularly the high levels of infection seen in southern Africa. Whatever factors are involved in HIV-1 spread in southern Africa, however, there is a great need to develop and test candidate vaccines that could stop new infections or ameliorate disease.

Among the most popular HIV vaccine candidates soon planned to enter or currently in human clinical trials are those designed to elicit virus-specific T-cell immune responses. Numerous studies have shown that virus-specific T cells play an important role in the control of viral replication (38, 41, 45). Vaccine strategies designed to elicit T-cell immune responses also appear to control viral replication or at least delay the onset of disease (2, 4, 5). Presentation of antigenic peptides to $CD8^+$ and $CD4^+$ T cells is restricted by major histocompatibility complex (MHC; also known as HLA) class I and class II molecules, respectively, which are expressed on the surfaces of antigen-presenting cells. MHC molecules are among the most polymorphic human gene products known. This diversity imposes a potential limitation on the widespread applicability of most current vaccine candidate designs, because these designs have, for the most part, been based on analysis of viral sequences and allele frequencies of MHC molecules in developed countries. We have previously shown, for example, that MHC class I allele frequencies in Botswana may differ substantially from those among different ethnic groups in North America, suggesting that information about common HLA alleles and haplotypes in a specific population of vaccinees may be valuable (33).

Certain MHC class II alleles have been associated with protection against HIV-1 infection (24) and/or better control of infection or rapid progression upon infection (7, 17, 20). Possession of certain MHC class II haplotypes has been shown to

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be associated with a better clinical outcome in acutely infected individuals receiving highly active antiretroviral therapy (25). The mechanism by which certain alleles confer resistance to HIV infection or better control of viral replication in infected individuals is not clearly understood, but it is plausible that accumulation of data from different populations may help resolve the mechanisms. However, most studies on allele frequencies of HLA class II molecules have been done with developed-country populations where HIV prevalence is low, and in many cases, serological methods were used for typing. There is an urgent need to extend these studies to countries most affected by the HIV epidemic, because intervention strategies must be designed for and tested in these populations. Molecular typing methods allow for more-precise allele identification and are more compatible with new nomenclature.

The main goal of the present study was to analyze the frequencies of HLA class II alleles in Botswana in order to better understand the role of MHC class II HLA alleles in HIV pathogenesis and to facilitate the design of an HLA-based HIV-1 vaccine for southern Africa. We postulated that, given the diversity seen in MHC class II alleles in the human population, class II frequencies in Botswana may differ from those documented for North American and European ethnic groups. This may, in turn, significantly affect the efficacy of vaccines designed to elicit $CD4^+$ T-helper cell responses. If so, the distribution and frequency of HLA alleles in the population should guide vaccine design and vaccine trial planning. We also hypothesized that certain HLA class II alleles may be associated with protection against infection or, upon infection, with better control of viremia or better immune function. The main aim of this study, therefore, was to determine the most common HLA class II allele frequencies in Botswana and to assess whether these frequencies are significantly different from those observed in Europe and North America. We also sought to analyze the frequencies of HLA class II alleles in the subsets of HIV-positive and HIV-negative individuals in Botswana. In addition, although the sample size does not provide high power, we used the data obtained to explore if there is an association of any MHC class II HLA allele with the level of plasma viral load (and CD4 count) as an indicator of a certain allele being associated with better control or rapid progression of HIV infection. Finally, we explored whether HLA class II heterozygosity may be associated with lower plasma viral load or high CD4 counts in infected study participants, as has been described for HLA class I alleles in other studies (6, 48).

MATERIALS AND METHODS

Study participants and sample preparation. Samples were collected from 55 HIV-negative and 74 HIV-positive consenting adults according to the guidelines of the institutional review boards of the Ministry of Health of Botswana and the Harvard School of Public Health. The participants were part of various Botswana–Harvard School of Public Health AIDS Initiative Partnership studies and the Botswana sentinel surveillance study. HIV serostatus was confirmed by two standard enzyme-linked immunosorbent assays (ELISA): Murex HIV-1.2.0 (Murex Biotech Limited, Dartford, United Kingdom) and Ortho HIV-1/HIV-2 Ab-Capture ELISA Test System (Ortho-Clinical Diagnostics, Inc., Raritan, N.J.). Viral load was determined using the automated COBAS Amplicor/Ampli Prep HIV-1 Monitor Test V1.5 (Roche Molecular Systems Inc., Branchburg, N.J.). CD4 cells were enumerated by using the Multitest kit (CD4/CD3/CD8/CD45) on a four-parameter FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, N.J.).

DNA for HLA typing was isolated from buffy coat or peripheral blood mononuclear cells using the QIAamp DNA Blood Mini kit (QIAGEN, Santa Clarita, Calif.) according to the manufacturer's instructions.

HLA typing. We performed molecular DNA typing of the MHC class II HLA alleles in the DRB and DQB1 loci. The HLA-DRB BigDye Terminator Sequencing-Based Typing kit (Applied Biosystems, Foster City, Calif.) was used for the DRB locus typing according to the manufacturer's instructions and in accordance with the guidelines established by the Committee for Quality Assurance and Standards of the American Society for Histocompatibility and Immunogenetics (3). Briefly, low-resolution sequence-specific primer PCR (SSP-PCR) was first performed based on allele group-specific motifs in the first hypervariable region of exon 2. The positive amplification reactions from the SSP-PCR were used as sequencing templates to generate the high-resolution allele typing information using BigDye terminator cycle sequencing chemistry on the ABI PRISM 3100 Genetic Analyzer automated sequencer (Applied Biosystems). Allele assignment was done using MatchTools software and MTNavigator software, provided in the Matchmaker Allele Identification software package.

For DQB locus genotyping, we used the low-resolution Micro SSP HLA DNA typing trays (One Lambda, Inc., Canoga Park, Calif.) or the DQB1 SSP UniTray kit (Pel-Freez Clinical Systems, LLC, Brown Deer, Wis.). These systems use SSP-PCR only. Typing results were interpreted according to the manufacturers' instructions.

Statistical analysis. The population frequency was estimated by weighting the HIV-positive and HIV-negative study participants so that they represented 35.4% and 64.6% of the population, respectively, which are the prevalences among antenatal-clinic women participating in the Botswana 2002 Second Generation HIV/AIDS Surveillance (29). Comparisons of HIV-infected and non-HIV-infected subjects were made using Fisher's exact test for binary variables and Fisher's exact test for $R \times C$ tables for categorical variables with more than two categories. CD4 cell counts and HIV-1 RNA viral load are summarized by their medians and compared using the Kruskal-Wallis test. Tests for a trend in the mean CD4 cell counts or HIV-1 RNA load by the number of alleles expressed were conducted by using an F-test from a linear regression model with the number of alleles as a continuous covariate in the model. Nominal P values without adjustment for multiple testing are presented.

To calculate 95% confidence intervals for the population frequency in Botswana, robust standard errors were first calculated in logistic regression models weighting the data to the population HIV seroprevalence, and then 95% confidence intervals were calculated on the log odds scale and transformed to the percentage scale. Ninety-five-percent confidence intervals for other populations were calculated using estimated frequency $\pm$ binomial standard errors multiplied by 1.96. Population frequencies in Botswana are compared to those in other populations as reported in the literature (10) using z-scores calculated from the difference in frequencies divided by its standard error.

RESULTS

Molecular DNA typing methods were used for DRB and DQB1 loci. However, resolution of specific four-or-more-digit alleles was possible for only 69 of 129 samples (approximately 53.5%), due to typing kit limitations. For DQB1, 118 of 125 samples were fully resolved to the specific allele level. Specific allele groups (two-digit designation) could be determined for all samples for DRB and DQB loci, and these are the results presented in this paper.

We first investigated the frequencies of allele groups expressed at the DRB1, DRB3, DRB4, and DRB5 loci within the DR region of the MHC class II gene cluster in HIV-positive and HIV-negative study participants. The results of this analysis are summarized in Table 1. Within the DRB1 locus, the allele groups DRB1*01, DRB1*03, DRB1*11, DRB1*13, and DRB1*15 (also known as DRB1*02) were all expressed at frequencies of 20% or more in this population. DRB1*11 was the most commonly expressed allele group in the DRB1 locus, with a population frequency of 35.5%. The majority of study participants (86.3%) expressed two allele groups at the DRB1 locus. Expression at the DRB3 locus was found for 81.1%, with 34.3% expressing DRB3*01, 38.8% expressing DRB3*02, and 27.4% expressing DRB3*03. Of those studied, 19.4% ex-

TABLE 1. HLA class II allele group expression frequencies at DRB and DQB1 loci[a] by HIV status

DRB allele group	Population frequency (%)	No. (%) of individuals with the indicated allele group[b]		P value[c]
		HIV$^+$ (n = 74)	HIV$^-$ (n = 55)	
DRB1*01	21.7	6 (8.1)	16 (29.1)	**0.002**
DRB1*02/15	21.3	15 (20.3)	12 (21.8)	0.83
DRB1*03	22.2	17 (23.0)	12 (21.8)	1.00
DRB1*04	14.4	13 (17.6)	7 (12.7)	0.62
DRB1*07	9.2	7 (9.5)	5 (9.1)	1.00
DRB1*08	14.2	10 (13.5)	8 (14.5)	1.00
DRB1*09	1.0	2 (2.7)	0 (0.0)	0.51
DRB1*10	6.1	3 (4.1)	4 (7.3)	0.46
DRB1*11	35.5	25 (33.8)	20 (36.4)	0.85
DRB1*12	7.6	6 (8.1)	4 (7.3)	1.00
DRB1*13	32.2	28 (37.8)	16 (29.1)	0.35
DRB1*14	0.5	1 (1.4)	0 (0.0)	1.00
DRB1*16	0.5	1 (1.4)	0 (0.0)	1.00
DRB3*01	34.3	25 (33.8)	19 (34.5)	1.00
DRB3*02	38.8	37 (50.0)	18 (32.7)	0.071
DRB3*03	27.4	18 (24.3)	16 (29.1)	0.55
DRB4*01	21.6	23 (31.1)	9 (16.4)	0.065
DRB5*01	19.4	11 (14.9)	12 (21.8)	0.36
DRB5*02	0.5	1 (1.4)	0 (0.0)	1.00
DQB1*02	14.9	14 (18.9)	7 (12.7)	0.47
DQB1*03	39.2	28 (37.8)	22 (40.0)	0.86
DQB1*04	25.8	22 (29.7)	13 (23.6)	0.12
DQB1*05	21.5	13 (17.6)	13 (23.6)	0.51
DQB1*06	47.7	31 (41.9)	28 (50.9)	0.37
DQB1 heterozygous[d]	51.8	37 (52.1)	29 (53.7)	1.00

[a] The DQB1 allele group was not determined for four study participants (three HIV positive and one HIV negative; 2.6%) because of lack of a DNA sample.
[b] Totals do not add up to 100% at DRB1 and DRB3 loci due to heterozygosity and at DRB5 due to rounding.
[c] By Fisher's exact test. The boldfaced value is significant.
[d] Study participants whose DQB1 allele groups could not be typed due to lack of a DNA sample were excluded from calculation of population frequencies and from the comparisons.

pressed two allele groups at the DRB3 locus. No DRB4 or DRB5 alleles were expressed by 78.4% or 80.2% of participants, respectively.

Within the DQB1 locus, DQB1*06 was the most common specificity in the population at 47.7%, while DQB1*03, DQB1*04, and DQB1*05 had population frequencies of 39.2%, 25.8%, and 21.5%, respectively (Table 1). DQB1 typing could not be done for four study participants (three HIV-positive and one HIV-negative individual; 2.6% of study participants) due to lack of a DNA sample. These samples were excluded from analyses of heterozygosity and analyses of numbers of genes expressed at DRB1, DRB3, DRB4, DRB5, and DQB1 loci.

HLA molecules have been independently associated with the susceptibility or resistance of individuals to HIV infection, and with favorable or unfavorable disease outcomes, although the mechanisms responsible for this are largely unknown. Table 1 summarizes the outcome of our investigation into whether there are differences in the frequency of expressed HLA class II genes between the HIV-positive and HIV-negative study participants. The only statistically significant difference between the two groups was that for the DRB1*01 allele group, which was overrepresented in the HIV-negative group compared to the HIV-positive group (29.1% versus 8.1% by Fisher's exact test; $P = 0.002$).

The availability of DRB1 and DQB1 data enabled us to estimate the most common DRB1–DQB1 two-locus haplotypes in the Botswana population (Table 2). Six two-locus haplotypes occurred at frequencies above 10%: DRB1*11–DQB1*03 (24.4%), DRB1*13–DQB1*06 (22.4%), DRB1*15–DQB1*06 (19.4%), DRB1*11–DQB1*06 (19.1%), DRB1*03–DQB1*04 (18%), and DRB1*04–DQB1*03 (11.8%). Two haplotypes that were expressed at overall frequencies above 5%, DRB1*01–DQB1*03 and DRB1*01–DQB1*04, were significantly more common among HIV-negative study participants than among HIV-positive participants, with respective P values of 0.013 and 0.042.

Further, we studied the association of HLA class II antigen specificities with plasma viral RNA loads and CD4 cell counts by comparing the median viral load (or CD4 cell count) of those expressing a particular HLA versus the rest of the population (Table 3). Within the DRB loci, only DRB1*08 was associated with a low median viral load ($P = 0.011$ by the Kruskal-Wallis test), while within the DQB1 locus, the expression of any particular antigen specificity did not seem to significantly affect median viral load or CD4 cell count. At the haplotype level, it has been shown previously that the DRB1*13–DQB1*06 haplotype is associated with an increased duration of AIDS-free time among homosexual men (21, 25). Among the study population, 22.4% expressed the DRB1*13–DQB1*06 haplotype, and we investigated whether possession of this haplotype would be associated with better control of viremia, as indicated by a low median viral load as a surrogate marker of a better clinical outcome. Possession of the DRB1*13–DQB06 haplotype was not associated with a lower median plasma viral load ($P = 0.63$), nor was there a statistically significant difference between HIV-positive and HIV-negative individuals among those who possessed this haplotype ($P = 0.67$) (data not shown).

We conducted a preliminary investigation of how HLA class II gene frequencies in the Botswana population may differ from those in well-studied populations such as those in North America, as well as those in other African countries or regions where HIV vaccine trials are likely to be undertaken. Figure 1 shows the population frequencies of the DRB1 allele groups in the Botswana population compared to those for North American Caucasians, African-Americans, black South Africans, black East Africans, and black West Africans. Statistically significant differences in population frequencies between the Botswana study population and North American Caucasians were found in the DRB1*04, DRB1*07, DRB1*08, DRB1*11, DRB1*13, and DRB1*14 allele groups. Significant differences were noted between Batswana and African-Americans in the DRB1*02/15, DRB1*07, DRB1*09, DRB1*11, and DRB1*14 specificities. Comparison between the Botswana study group and other black African populations was somewhat limited because data were unavailable for the other populations for allele groups DRB1*01, DRB1*04, DRB1*07, DRB1*08, DRB1*09, and DRB1*10. Comparisons to other African populations are also limited by small sample sizes of the other studies as well as of ours. However, DRB1*11 appears to be the most common allele group among all the African groups, at 35.5% among Batswana and 41.0%, 45.5%, and 38.8% among

TABLE 2. Population expression frequency of DRB1/DQB1 haplotypes and comparison by HIV status[a]

DRB1–DQB1 haplotype expressed	Population frequency[b] (95% confidence interval)	No. (%) of individuals with the indicated allele groups		P value[c]
		HIV+ (n = 74)	HIV− (n = 55)	
DRB1*01–DQB1*03	5.9 (2.5, 13.3)	0 (0.0)	5 (9.1)	**0.013**
DRB1*01–DQB1*04	7.5 (3.6, 15.2)	1 (1.4)	6 (10.9)	**0.042**
DRB1*01–DQB1*05	7.3 (3.5, 14.5)	3 (4.1)	5 (9.1)	0.28
DRB1*01–DQB1*06	5.4 (2.4, 11.7)	4 (5.4)	3 (5.5)	1.00
DRB1*03–DQB1*04	18.0 (11.8, 26.4)	13 (17.6)	10 (18.2)	1.00
DRB1*03–DQB1*06	5.7 (2.8, 11.3)	7 (9.5)	2 (3.6)	0.30
DRB1*04–DQB1*03	11.8 (7.0, 19.3)	10 (13.5)	6 (10.9)	0.79
DRB1*07–DQB1*02	8.0 (4.2, 14.8)	7 (9.5)	4 (7.3)	0.76
DRB1*08–DQB1*03	9.0 (4.7, 16.5)	4 (5.4)	6 (10.9)	0.32
DRB1*08–DQB1*04	6.4 (3.1, 12.6)	6 (8.1)	3 (5.5)	0.73
DRB1*10–DQB1*05	5.7 (2.4, 12.5)	2 (2.7)	4 (7.3)	0.40
DRB1*11–DQB1*03	24.4 (17.2, 33.3)	19 (25.7)	13 (23.6)	0.84
DRB1*11–DQB1*05	5.0 (2.1, 11.3)	3 (4.1)	3 (5.5)	0.70
DRB1*11–DQB1*06	19.1 (12.5, 28.1)	8 (10.8)	13 (23.6)	0.058
DRB1*12–DQB1*05	5.0 (2.1, 11.3)	3 (4.1)	3 (5.5)	0.70
DRB1*12–DQB1*06	6.4 (3.1, 12.6)	6 (8.1)	3 (5.5)	0.73
DRB1*13–DQB1*03	7.4 (3.9, 13.6)	8 (10.8)	3 (5.5)	0.35
DRB1*13–DQB1*04	5.0 (2.5, 9.9)	8 (10.8)	1 (1.8)	0.077
DRB1*13–DQB1*05	8.7 (4.6, 15.9)	6 (8.1)	5 (9.1)	1.00
DRB1*13–DQB1*06	22.4 (15.5, 31.4)	15 (20.3)	13 (23.6)	0.67
DRB1*15–DQB1*03	7.3 (3.5, 14.5)	3 (4.1)	5 (9.1)	0.28
DRB1*15–DQB1*06	19.4 (12.8, 28.2)	11 (14.9)	12 (21.8)	0.36

[a] Heterozygous expression at DRB1 was found for 86.3% of individuals, so these individuals may contribute twice to these frequencies. For example, if an individual expresses DRB1*01/03 and DQB1*05, that individual contributes to the DRB1*01–DQB1*05 and DRB1*03–DQB1*05 haplotype frequencies.
[b] Expressed as a percentage.
[c] Significant values are boldfaced.

black South Africans, East Africans, and West Africans, respectively. There was no significant difference between these African groups in this locus (Fig. 1i). DRB1*13 is also commonly expressed among Africans at 32.2% (Batswana), 27.6% (black South Africans), 28.5% (East Africans), and 19.0% (West Africans). These population frequencies did not translate into significant differences between the populations (Fig. 1k). Significant population frequency differences were found

TABLE 3. Comparison of $CD4^+$ cell counts and HIV-1 RNA loads by HLA class II allele group expression at DRB1 and DQB1 loci among HIV-infected Batswana[a]

DRB1/DQB1 allele group	Median $CD4^+$ cell count[b] (no. of individuals) where the indicated allele group is:		P value[c] for $CD4^+$ cell count	Median HIV-1 RNA load[d] (no. of individuals) where the indicated allele group is:		P value[c] for HIV-1 RNA load
	Expressed	Not expressed		Expressed	Not expressed	
DRB1*01	392 (6)	348 (57)	0.59	4.9 (5)	4.7 (59)	0.55
DRB1*02/15	374 (12)	344 (51)	0.69	4.7 (13)	4.8 (51)	0.89
DRB1*03	311 (13)	390 (50)	0.10	5.1 (17)	4.7 (47)	0.47
DRB1*04	400 (11)	346 (52)	0.45	5.1 (9)	4.8 (55)	0.41
DRB1*07	236 (5)	365 (58)	0.30	4.4 (5)	4.8 (59)	0.49
DRB1*08	434 (10)	317 (53)	0.091	3.3 (7)	4.8 (57)	**0.011**
DRB1*09	317 (2)	351 (61)	0.72	4.8 (2)	4.7 (62)	0.94
DRB1*10	314 (3)	350 (60)	0.85	4.4 (3)	4.8 (61)	0.94
DRB1*11	379 (19)	335 (44)	0.74	4.8 (22)	4.6 (42)	0.41
DRB1*12	366 (6)	348 (57)	1.00	3.7 (6)	4.8 (58)	0.21
DRB1*13	379 (25)	337 (38)	0.81	4.7 (24)	4.8 (40)	0.92
DRB1*14	448 (1)	346 (62)	0.51	4.6 (1)	4.8 (63)	0.77
DRB1*16	7 (1)	350 (62)	0.11	5.9 (1)	4.7 (63)	0.11
DQB1*02	317 (12)	385 (51)	0.41	4.6 (10)	4.8 (54)	0.34
DQB1*03	400 (21)	319 (42)	0.26	4.9 (21)	4.7 (43)	0.57
DQB1*04	320 (19)	365 (44)	0.40	4.9 (20)	4.8 (44)	0.98
DQB1*05	391 (12)	344 (51)	0.95	4.8 (12)	4.7 (52)	0.60
DQB1*06	309 (28)	379 (35)	0.21	4.6 (29)	4.8 (35)	0.58
DQB1 unknown	448 (3)	346 (60)	0.59	4.7 (3)	4.8 (61)	0.47

[a] CD4 cell count and plasma viral load data were unavailable for 11 and 10 HIV-positive study participants, respectively.
[b] Expressed as number of cells per cubic millimeter.
[c] By the Kruskal-Wallis test. Significant values are boldfaced.
[d] Expressed as log copies per milliliter.

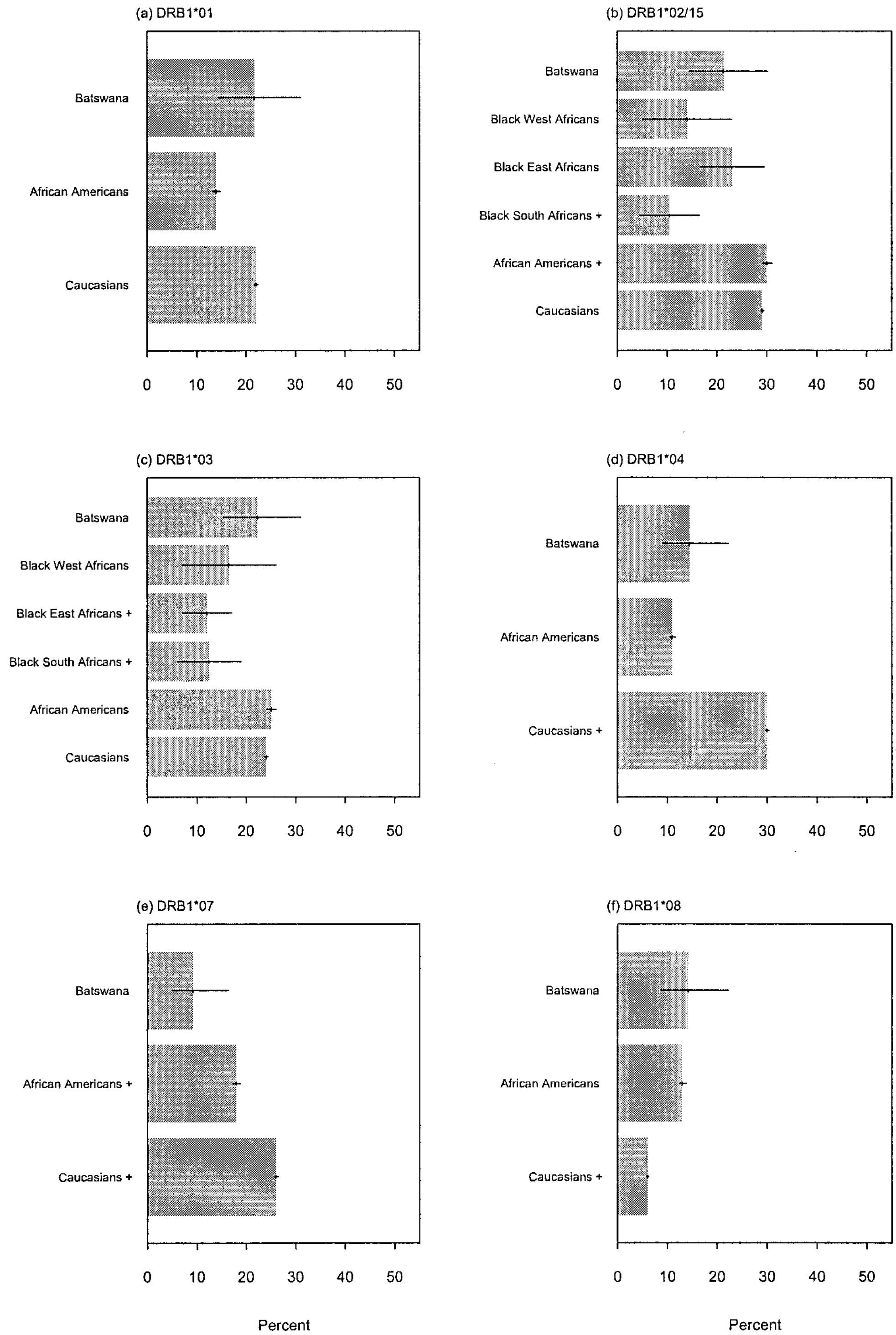

FIG. 1. Botswana DRB1 allele group frequencies compared with those of other populations. The data for the African populations are taken from the work of Dunand et al. (10) and this study (for Botswana), while those for North American Caucasians and African-Americans are taken from the work of Sintasath et al. (46). Data were not available for black West Africans, East Africans, and South Africans for allele groups DRB1*01, DRB1*04, and DRB1*07 to DRB1*10. The horizontal line represents the calculated 95% confidence interval for each population frequency. Plus signs indicate population frequencies that are significantly different from those for Botswana ($P < 0.05$).

between Batswana and black South Africans in the DRB1*02/15 allele group (Fig. 1b) and among Batswana, South Africans, and East Africans in the DRB1*03 (Fig. 1c) and DRB1*14 (Fig. 1l) allele groups.

HLA class I heterozygosity has been associated with better control of HIV-1 infection (6, 48). We investigated whether HLA class II heterozygosity at any of the HLA class II loci studied might likewise be associated with a better outcome, as

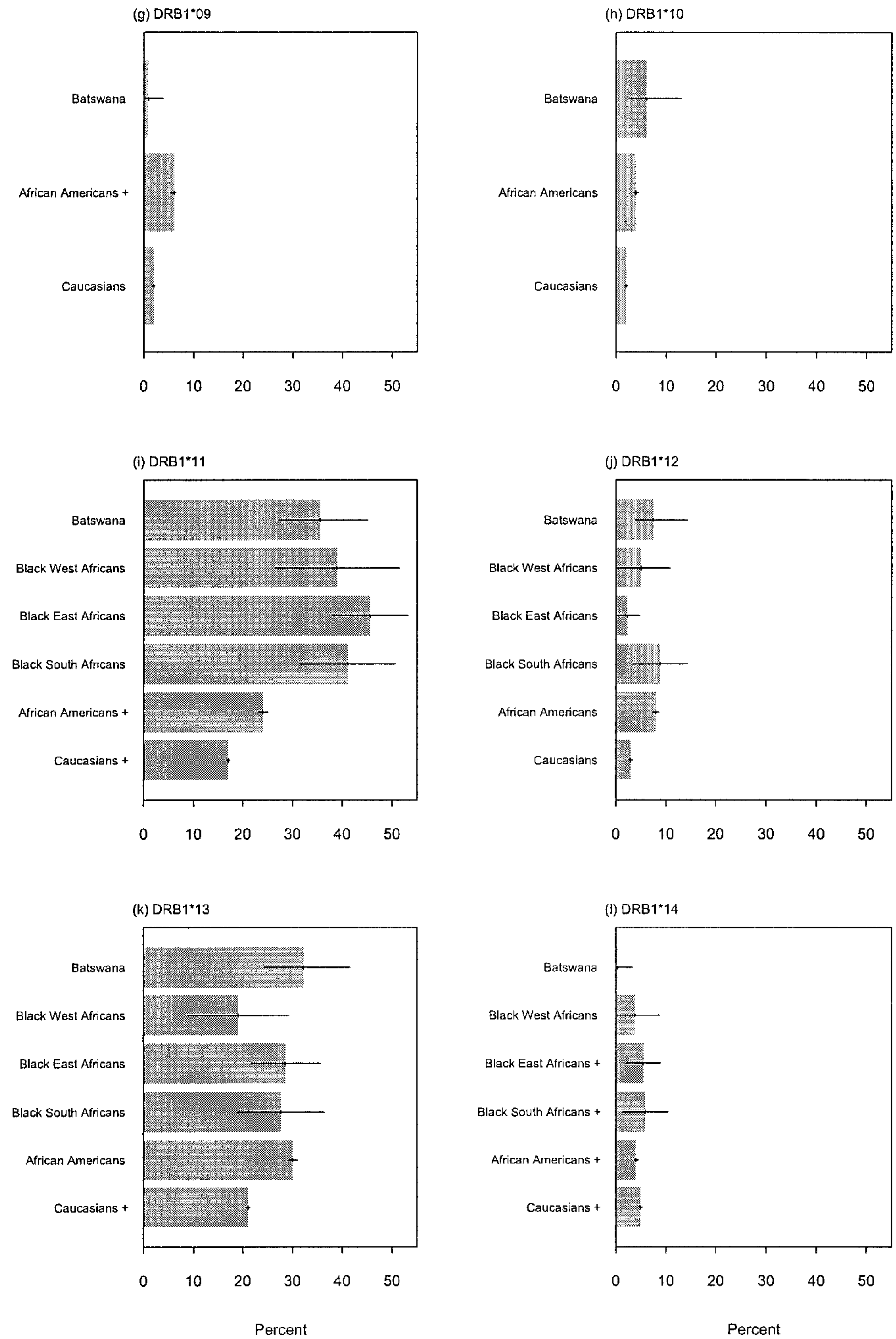

FIG. 1—*Continued.*

suggested by a tendency toward lower viral load and/or higher CD4 cell counts. No association was found between heterozygozity and low viral load or high CD4 cell counts. We applied a trend test that assumes normality to assess whether the total number of DRB and DQB alleles expressed by an individual, when taken together, was associated with a low median viral load or a high CD4 cell count, but the *P* values of 0.84 and 0.20, respectively, did not reach statistical significance (data not shown).

DISCUSSION

In this study, we identified the population frequencies of different MHC class II HLA allele groups in Botswana. We identified DRB1*11, DRB1*13, DRB1*03, DRB1*01, and

DRB1*02/15 as the most commonly expressed DRB1 allele groups in Botswana, all occurring at frequencies above 20% in the study population. DQB1*06 was the most commonly expressed allele group at the DQB1 locus, with 47.7% of the study population expressing it, followed by DQB1*03, DQB1*04, and DQB1*05 at 39.2%, 25.8%, and 21.5%, respectively. This is the first study to investigate the relative frequencies of HLA class II gene expression in Botswana, a country at the epicenter of the HIV/AIDS epidemic. This study addresses directly the problem of lack of information on generic HLA types in different populations, which is a significant obstacle in the design of an HLA-based HIV vaccine (51). Although the numbers in this study are small and may need to be extended to define the most common alleles within each allele group, this study provides evidence that by targeting the most commonly encountered HLA allele groups within a specific region or population, it may be possible, at least in part, to overcome the problem of HLA polymorphism, which is likely to affect the widespread efficacy of HIV vaccines designed to elicit virus-specific T-cell responses. A particularly interesting finding from this study is that the population frequency of some commonly expressed allele groups, such as DRB1*11 and DRB1*13, approximates that in other African populations (48). This suggests that by targeting these commonly expressed HLA class II alleles in HIV vaccine design strategies, it may be possible to overcome problems associated with genetic diversity within populations where HIV vaccines are urgently needed. Obviously, studies of HLA class II allele frequencies and distribution will need to be extended elsewhere in areas with high HIV prevalence and incidence rates, especially in sub-Saharan Africa, in order to define the extent of commonalities between populations. The significant differences found between our study population and well-studied populations in North America (such as in allele group specificities DRB1*11 and DRB1*13, which are those most commonly encountered in Botswana) suggest that disregarding local HLA gene frequencies in vaccine design may compromise the efficacy of a vaccine in the populations most likely to need it.

Numerous studies have previously shown that certain HLA genotypes may be associated with increased susceptibility or resistance to HIV-1 infection. Other HLA genotypes are associated with slow or rapid progression to AIDS. In this study, we found that the frequency of DRB1*01 was higher among HIV-negative than among HIV-positive people, suggesting that this genotype may be protective against HIV infection. This finding is in agreement with data from MacDonald et al. for a longitudinal cohort of female commercial sex workers in Kenya (24). The mechanism of this protection remains unidentified, but our findings lend further support to the suggestions that $CD4^+$ T-helper cells may play an important role in protection against HIV-1 infection and are necessary for maintaining an effective cytolytic T-lymphocyte response in the course of HIV infection (1, 19). Because induction of HIV-specific cytolytic T-lymphocyte and T-helper responses is an important goal in the development of an AIDS vaccine (15, 16), further studies are needed to address the mechanisms involved, which could include direct cytolytic activity (23) or the action of cytotoxic $CD8^+$ T cells (27). Another interesting finding in this study is the suggested association of DRB1*08 with a lower median viral load. These results need to be interpreted cautiously, because a large number of statistical tests were conducted to explore the relationship of allele groups with HIV infection status, $CD4^+$ T-cell count, and viral load using the nominal 5% false-positive benchmark. The increased rate of false-positive results when multiple testing is performed is well known, and results here need to be confirmed in other independent studies. If confirmed, the observation that the alleles associated with protection may be different from those associated with lower median viral load may suggest that the mechanisms (or at least the T epitopes) associated with protection may be different from those that control an established viral infection. This is an important issue with potentially serious implications for HIV-1 vaccine design and needs to be addressed in a larger prospective longitudinal cohort study. It should also be noted that this study did not find evidence for differences in median $CD4^+$ cell count or viral load for individuals with the DRB1*13–DQB6 haplotype, which has been associated with slower progression in North American studies. Again, this result needs to be interpreted with caution, because the times of infection in our study were unknown, early progressors may not be represented, and the small sample size limited the power of our analysis.

This study is an extension of a series of studies we have undertaken in the recent past in an attempt to understand the biological determinants that may be behind the epidemic in Botswana and southern Africa. We have previously shown that HIV-1 subtype C is the predominant subtype in Botswana and have undertaken detailed biological and genetic characterization of this virus (30, 31, 35, 37). Genetic diversity within HIV-1 is widely appreciated as a major obstacle to the development of an efficacious HIV-1 vaccine. We have suggested the use of consensus sequences as one possible approach to reducing the challenge of HIV-1 diversity in vaccine design. We have also identified the most immunogenic regions across viral proteins, and the T-cell responses associated with low viral load in HIV-1 subtype C infection in Botswana (32, 34, 36). Previously, we reported on the most common HLA class I specificities in Botswana (33). Further longitudinal studies will be needed to tease out the kinetics of breadth, the hierarchy and durability of immune responses, and the identification of responses associated with protection against HIV-1 infection and progression to AIDS. In the meantime, the information described here may assist in rational approaches to vaccine design for southern Africa in general and Botswana in particular.

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